Autoflex Speed is a MALDI Operating Instructions

Background

The Autoflex Speed is a MALDI (Matrix Assisted Laser Desorption/Ionization) mass spectrometer designed for high-throughput automated MS and MS/MS identification of proteins and peptides. The Autoflex Speed also boasts as a TOF/TOF (Time of Flight) tandem mass spectrometer. The Autoflex is an innovative MALDI-TOF technology that is designed and optimized to be easy-to-use system for traditional MALDI applications.

Safety

- Care should be taken to prevent the MALDI target plate holder from being damaged as this piece is vital for operation of the machine.
- Carefully take the target plate out of its plastic housing and place it back after use.
- Care should be taken that area surrounding the MALDI's sample loading tray is free of any obstructions, including yourself.

Before Using the Machine (!)

- New Users: Email Steven Guillen (steven.guillen@usc.edu) to create an account for CNHS use, data storage, training, and maintenance.
- New Users: Email Dr. Joo-Ho Lee (jooholee@usc.edu) to create a passcode for the door.
- Check FBS to see the status of the machine before use; reserve time for use on the machine.
- Please record your use of the machine using the provided logbook and any issues with the machine as they arise.

Contents

Setting up the Machine

- Log into the MALDI-controlled computer using your nmrnet username and password.

- Click the **Auto Flex Control** to start the software. Allow some time for the background processes to load the instrument parameters via the software.

Selecting a Method
- **Click File > Select Method**. A window will open and will show all existing preset methods.

> **Note** Here you will find different modes like choosing between positive or negative, reflectance, or mass range in daltons (D) or kilodaltons (kD).

- Select a suitable method file and click **Open**. The files names are categorized according to choice of detection mode, polarity, and mass range.
  - ➢ **Example** Opening the method, "LP_700-2000Da", provides a linear positive mode in which positive ions are detected in the mass range of 700-2000 Da.

> **Note** You can change the parameters to suit your particular sample needs, but in doing so you will need to Save this new (i.e., an altered method) under a different name in your directory.

> **Note** L = Linear, R = Reflector, P = Positive Ion Mode, and N = Negative Ion Mode III.

Preparing Your Sample
- All samples should be prepared according to your experimental needs. Here are a few tips to aid in best preparing your samples for MALDI use —
  1. All samples should be placed around the calibration standard or very close.
  2. For all samples, the "flower shape" will be the best way to spot them on your plate.
  3. Do not spot your standard and samples on the edges of your sample.
  4. The plate is big and samples should be placed in the middle of the plate around the standard (i.e., the "flower shape").

Loading Your Sample Plate
- Place the target plate with your prepared samples on the target plate holder. The target plate holder is located in the desk drawer and must be placed back when finished as a courtesy for the next user.

> **Note** This target plate holder is **very** expensive and should be treated very carefully to avoid downtime and unnecessary expenses on the MADLI machine.

- The target plate holder has a flat handle on the bottom which locks your target plate on the top. This lock will make contact with three points on the frame. There should be no gaps between the target plate holder and the target plate. Make sure that the plate fits snugly.
- On the MALDI, push the **Eject/Load** (green-square button). The sample door will open and the sample tray for your target plate will come out.

> **Note** The tray and loading mechanism is very fragile. Please do not bump, or apply any force on the tray. Always keeps the area around the sample door clear of any obstructions, including yourself.

- Place the target plate holder with your attached sample target plate on the sample tray so the beveled edges are pointing towards the MALDI. Press **Eject/Load** to load the tray in the machine for analysis.

Running Your Sample Plate (Firing the Laser)

- If needed, adjust the **Number of Slots** (e.g., default = 30 slots, but each method is adjusted already) by changing the value in a small window below the camera screen. Use the slide bar (to the right of the camera screen) to adjust the **Laser's Power**. A good starting point is ~20% power.
- It will take a few minutes for the target plate to load and for the system to reach proper vacuum. The **Sample Loading** is indicated by on the software screen by a **yellow** button turning **green** on the bottom right corner. The laser indicator is found immediately left to the Sample Loading button.
- Point the cursor on the laser indicator and hold until it shows **Laser on Standby**, if this is not shown, then the laster will still be ramping up to be ready to be on standby. The laser indicator will remain **yellow** until you fire the laser for the first time, then it will turn **green** with a **Ready** sign.
- Click the **Start** button to fire the laser. The spectrum will be displayed on the screen.

Note The laser power can be adjusted for the spectrum to be acquired in higher intensity if needed, but at the same times this may lead to excessive laser power. Therefore, adjust the laser power and fire at the same spot location to try and best achieve the highest quality spectrum possible.

✶ Examine the spectrum for quality. Is the calibration peak(s) present? Is the signal intensity too high? Too weak? If so, adjustments to the laser power may be needed, or a different crystal/sample spot may need to be selected. Any adjustments to these parameters will result in a new spectrum needed to be.

Calibrating the Machine

- Calibration is required whenever a plate or method is changed, and is therefore suggested every time a user uses the machine. To calibrate the machine, click on the **Calibration** tab. Select the calibrant from the drop-down menu and select one from the preset lists of standards.

Note You, as the user, can manually enter values for the calibrant by entering the appropriate values into the boxes below the calibrant list.

- To enter calibrant information manually, click the **Edit** button and enter the name, mass, and the error range (in parts per million, ppm; ~500 ppm is okay).
- Click **Start** to get the spectrum and then highlight (or select) your listed calibrant line. A **red** line will appear on the spectrum at the expected m/z the respective calibrant.
- Select the calibrant on the spectrum by left-clicking on the left of the calibrant peak on the spectrum. By doing this, the system will select the peak. Then, the **red** line will turn **green** and a small **white** arrow will indicate the selected peak.
- Click **Apply** to calibrate the spectrum.
  - ➢ If needed, select another calibrant and repeat the calibration procedure for other calibrant peaks as necessary. Click **Apply** to calibrate the spectrum. The instrument will now be calibrated and this calibration will be applied to all spectra until another method file is opened, or the target is removed, or the software is closed.
- Click on the **Sample Carrier** tab and select a sample spot containing the calibrant to be analyzed. Click the **Start** button to start acquiring the data, and again adjust the laser power to achieve the best possible analysis quality.

Saving or Printing Your Data
- To save your data, click **File > Save As** and place the file within your respective data folder.
- A region of interest in the resulting spectrum can be enlarged using the mouse to select, or by clicking **Zoom** on the tool bar.
- Peaks can be labeled by clicking the **Peak Label** button on the toolbar.
- The spectrum can be printed by clicking the **Printer** button on the toolbar.

Turning Off The MALDI
- After completing your study, press the **Eject/Load** button on the side of the MALDI or click the Eject button within the software. The MALDI's sample door will open and the sample tray, with the sample target plate and holder, will be ejected.
- Remove your sample target plate and the holder and immediately press the **Eject/Load** button again to retract the tray and close the door.

Note The sample loading tray is very sensitive and may get damaged with even the slightest impact. Therefore, be cautious and close the sample door as soon as you remove your sample.

- Separate your sample target plate from the target plate holder by releasing the handle located on the backside of the target plate holder. The adapter handle will release its pins such that your target plate can be easily separated.
- Exit the Auto Flex Control software program.
- Patiently wait until the **Log Off** button turns from gray to black, and only then click on **Log Off**.

Transferring Data from Data Analysis to MNOVA or How to Open MALDI Data Files Using MNOVA
- Log into the MALDI-controlled computer using your nmrnet username and password.
- Open the Auto Flex Data Analysis software program.
- **Export** your data file as an **.mzXML** file and save it onto your data folder that connects to nmrnet. The .mzXML file can now be read by MNOVA software; MNOVA can be downloaded from nmrnet.